

NOTES

The reduction of root colonization by mycorrhizal fungi by mycophagous amoebae

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Two mycophagous amoebae, *Saccamoeba* sp. and *Gephyramoeba* sp., reduced the colonization of pine roots in soil by the ectomycorrhizal fungus, *Rhizopogon luteolus*, when added at the same time as the fungus or 2 weeks later. Another mycophagous amoeba, an unidentified leptomyxid, was unable to do so. When seedlings were grown in sterilized soil amended with different population levels of *Saccamoeba*, 42 and 85 amoebae $\cdot$ g soil⁻¹ had no effect but large reductions in root colonization by *R. luteolus* occurred when 430 and 860 amoebae $\cdot$ g soil⁻¹ were added. After 4 weeks, however, the protozoa population increased to a similarly high level in all treatments irrespective of the numbers added initially. Our results indicate that protozoa can have a large effect on the establishment of inoculated symbiotic fungi and could markedly affect the microbial composition of the rhizosphere.

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Deux amibes mycophages, *Saccamoeba* sp. et *Gephyramoeba* sp., ont réduit la colonisation de racines de pins en sol par le *Rhizopogon luteolus*, un champignon ectomycorhizateur, lorsque ces amibes étaient ajoutées au sol en même temps que le champignon, ou 2 semaines plus tard. Une autre amibe mycophage, une leptomyxide non identifiée, ne s'est pas comportée de la même façon. Lorsque des plantules étaient empotées en sol stérile amendé avec différents niveaux de population de *Saccamoeba*, 42 et 85 amibes g de sol⁻¹ n'ont eu aucun effet; mais lorsque 430 et 860 amibes g de sol⁻¹ furent ajoutées, la colonisation des racines par *R. luteolus* a subi des réductions importantes. Après 4 semaines, cependant, les populations de protozoaires ont atteint un niveau élevé similaire dans tous les essais, sans égard aux nombres d'amibes ajoutés initialement. Ces résultats indiquent que les protozoaires peuvent avoir un effet important sur l'établissement de symbiotes fongiques inoculés et pourraient affecter de façon marquée la composition microbienne de la rhizosphère.

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Mycophagous amoebae have been isolated from soils of several countries (Old and Patrick 1979; Chakraborty 1983) and are frequent colonizers of the rhizosphere. Rouatt *et al.* (1960) found significantly higher populations of protozoa in the rhizosphere of spring wheat than in the corresponding root-free soils, and subsequently large populations of amoebae have been reported in the rhizospheres of wheat (Geltzer 1963), white mustard, clover, and perennial rye grass (Darbyshire and Greaves 1973), beach grass (Napolitano 1983), and spring barley infected with take-all disease (Darbyshire *et al.* 1977). Soils suppressive to the wheat take-all fungus can also support higher rhizosphere populations of mycophagous (and other) amoebae than do nonsuppressive soils (Chakraborty 1983). Mycophagous amoebae can reduce the inoculum potential of plant pathogenic fungi (Chakraborty and Warcup 1983) and also reduce the severity of the diseases that the fungi cause (Chakraborty and Warcup 1984a). Many mycophagous amoebae can feed on a wide range of fungal species including vesicular-arbuscular and ectomycorrhizal fungi (Old and Patrick 1979).

The role of mycophagous amoebae in terrestrial ecosystems is only marginally understood. Their ability to reduce the inoculum potential of a variety of soil fungi, their survival and growth in the highly competitive environment of the rhizosphere, and the broad range of fungal prey warrant thorough investigation on their possible importance in soil fungus — plant interactions. We report the effect of three mycophagous amoebae on the colonization of *Pinus radiata* roots by the ectomycorrhizal fungus *Rhizopogon luteolus*.

The effects of three mycophagous amoebae, *Saccamoeba*

sp., *Gephyramoeba* sp., and an unidentified leptomyxid amoeba, on fungus colonization of the root were studied using the method of Theodorou and Bowen (1969). Mount Burr sand (an orthic podzol (Anonymous 1978), a forest soil) was moistened to -8 kPa (70% field capacity), dispensed in 60-g lots to cotton-plugged test tubes (20 $\times$ 3 cm), and sterilized by autoclaving. Two sterile seedlings of *P. radiata* with radicles 1 cm long were placed in each of 15 replicate tubes per treatment. The seedlings were inoculated with the ectomycorrhizal fungus *R. luteolus* by placing a 5 mm diameter Melin-Norkrans (Melin 1959) agar plug colonized by the fungus (washed to remove nutrients from the agar) beside the radicle. The amoebae were grown in the presence of a *Klebsiella* sp. as a food source and the amoebae (plus *Klebsiella*) were suspended in Neff's modified amoeba saline. Twelve millilitres of the suspension were applied around the root (final soil water potential, -7 kPa). Amoebae (plus bacteria) were added alone and together. Two controls were used: sterile amoeba saline and a suspension of *Klebsiella* in the saline. In a further treatment the fungus was allowed to colonize the root for 2 weeks before the mixture of amoebae was added. The tubes were randomized in a growth chamber at 12-h day (with illuminance of 16.14 klx) and 24:16°C (day:night) temperatures. Plants were harvested 4 weeks later. The roots were gently washed with sterile distilled water to dislodge the soil, then stained with 1% cotton blue in lactophenol for 1 min, and subsequently examined for length colonized by the fungus as well as intensity of colonization. The intensity was a rating at the region of greatest fungal growth on a 0–4 scale (0 = no growth, 1 = slight, 2 = moderate, 3 = heavy, 4 = very heavy) (Theodorou and

Bowen 1969).

The *Klebsiella* alone reduced fungal colonization by 40% (significant at $P < 0.001$) (see Table 1). The unidentified leptomyxid did not reduce it further but *Saccamoeba* sp. and *Gephyramoeba* sp. reduced fungal colonization by 71 and 54%, respectively, of colonization in the presence of *Klebsiella* alone (significant at $P < 0.001$). A mixture of amoebae significantly reduced colonization. In the treatment in which the amoeba mixture was introduced at 2 weeks the subsequent rate of fungus colonization was identical to that when *Saccamoeba* sp. were present from the commencement of the experiment (0.17–0.18 versus 0.58 mm·day⁻¹ with *Klebsiella* alone). This confirms that the amoebae exerted their effect during root colonization by the fungus, not *via* feeding on the inoculum plug. Other bacteria are known to reduce root colonization by mycorrhizal fungi (Bowen and Theodorou 1979) presumably by competition for nutrients, while some antagonistic bacteria reduced colonization to that obtained with *Saccamoeba* sp. and *Gephyramoeba* sp.

In the first experiment a high inoculum level of amoebae was applied with the fungal inoculum. A second experiment simulated a field situation more closely, in which the fungus was inoculated in the same way but the *Saccamoeba* sp. was distributed through the soil at 42, 85, 423, and 846 amoebae·g soil⁻¹. After 4 weeks in the growth cabinet, plants were harvested and examined for root colonization. Three replicate samples of rhizosphere and bulk soil were plated in a 10-fold dilution series (Chakraborty and Warcup 1984b) to determine the final population of *Saccamoeba*. *Saccamoeba* sp. significantly reduced colonization by *R. luteolus* at the two higher population levels (Table 2) but at the two lower concentrations the reduction was not significantly different from that obtained with the *Klebsiella* alone. Populations of up to several hundred *Saccamoeba* sp. per gram soil are common in soils containing high organic matter (Chakraborty and Warcup 1984b). Therefore it is evident that natural populations of this amoeba could significantly reduce root colonization by *R. luteolus*. In laboratory media (Chakraborty *et al.* 1983) the three amoebae used differ in their range of fungal species utilized as food. The inability of the mycophagous leptomyxid isolate to reduce the colonization of *R. luteolus* further points to specificity of some mycophagous amoebae for their fungal preys. Similar specificity has been reported for the mycophagous amoeba *Cashia mycophaga* (Pussard *et al.* 1980).

One interesting aspect of the results was the slight but significant increase in the root length obtained in treatments where

TABLE 1. Colonization of *P. radiata* roots by *R. luteolus* in the presence of amoebae

Treatment	Root length (mm)	Length of root colonized (mm)	Rate of colonization (mm·day ⁻¹)	Intensity (ratings, 0–4)*
<i>R. luteolus</i>	80a	30.5a	0.95	3.2a
<i>R. luteolus</i> + <i>Klebsiella</i> sp.	81a	18.4c	0.58	2.2c
<i>R. luteolus</i> + <i>Saccamoeba</i> sp.	82a	5.3b	0.17	0.9b
<i>R. luteolus</i> + <i>Gephyramoeba</i> sp.	77a	8.4b,d	0.26	1.2b
<i>R. luteolus</i> + unidentified leptomyxid	80a	17.8c	0.56	2.0c
<i>R. luteolus</i> + mixture of the three amoebae	82a	9.4d	0.29	1.1b
<i>R. luteolus</i> Mixture added 2 weeks later	80	16.1	0.18	1.9
<i>R. luteolus</i> 0–2 weeks growth	47	13.6	0.97	2.1
2–4 weeks (calculated)	33	16.9	1.2	—

NOTE: Data followed by a different letter are significantly different at $P < 0.001$.

*Rating 0–4, 0 = no growth, 1 = slight, 2 = moderate, 3 = heavy, 4 = very heavy.

either *Klebsiella* or *Saccamoeba* with *Klebsiella* were present (Table 2).

Saccamoeba grew both in the rhizosphere of *P. radiata* and in the soil, and the population increased within the 4 weeks irrespective of the initial population size (Table 3). *Saccamoeba* sp. with a rhizosphere and soil ratio of up to 20:1 appears to be as effective as some bacteria, as a rhizosphere colonizer.

Mycophagous amoebae have been proposed as naturally occurring biological control agents for plant pathogenic soil fungi (Old and Patrick 1979; S. Chakraborty, 1984, Ph.D. thesis, University of Adelaide). Bowen (1980) indicated the need to examine microbial interactions experimentally in soil ecosystems, and postulated that the predator–prey relationships of soil mesofauna in the two dimensional surface of the root would be quite different from that of interactions in soil volumes. This study indicates the potential importance of protozoa

TABLE 2. Colonization of *P. radiata* roots by *R. luteolus* in the presence of different numbers of *Saccamoeba* sp.

Treatment	No. of <i>Saccamoeba</i> sp. · g soil ⁻¹	Root length (mm)	Length of root colonized (mm)
<i>R. luteolus</i>	—	89a	23.6a
<i>R. luteolus</i> + <i>Klebsiella</i> sp.	—	100b	12.9b
<i>R. luteolus</i> + <i>Saccamoeba</i> sp.	846	100b	5.8c
	423	98b	8.0c
	85	102b	11.3b
	42	103b	12.9b

NOTE: Data followed by different letters are significantly different at $P < 0.01$.

TABLE 3. Population of *Saccamoeba* sp. (as number of amoebae per gram of soil) after 4 weeks growth in the presence of pine roots and *R. luteolus*

Initial population	Final population		
	Rhizosphere (R)	Nonrhizosphere (S)	R/S ratio
846	14817	730	20:1
423	9048	908	10:1
85	7190	738	10:1
42	21164	1182	18:1

in influencing the establishment of inoculated symbiotic fungi and in affecting the microbial composition of the rhizosphere and points to the need for research in this area. In this study we restricted ourselves to examining root colonization by the ectomycorrhizal fungus; the marked reduction of fungal spread caused by some amoebae would be expected to depress mycorrhiza production over the root system. Amoebae would also reduce the penetration of soil by hyphae from the mycorrhiza, one of the main ways in which mycorrhizal fungi increase nutrient uptake (Bowen 1973). This study also supports the concept (Bowen 1980) that biological control of symbionts is just as real a phenomenon as that of pathogens and must be considered in designing management systems that enhance the introduction and proliferation of symbionts.

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